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Two striking *Inocybe* species from Yunnan Province, China

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Abstract —Two striking species, *Inocybe caroticolor* sp. nov. and *I. olivaceonigra,* are documented in this paper. *Inocybe caroticolor* is characterized by its carrot-colored basidiomata, entirely pruinose stipe, aromatic odor, and nodulose basidiospores. *Inocybe olivaceonigra*, originally described from Papua New Guinea, is new to China. Illustrated descriptions of the two species are provided based on the Chinese collections. DNA sequences of internal transcribed spacer regions (ITS) of the Chinese materials were generated, analyzed, and submitted to the GenBank.

Key words — *Agaricales, Inocybaceae,* taxonomy

Introduction

Inocybe (Fr.) Fr. is a large agaric genus, which was established as a "tribe" of *Agaricus* by Fries in 1821 and elevated to genus rank in 1863. Currently, there are about 500 (Kirk et al. 2008) to 700 (Matheny et al. 2009) species of *Inocybe* recognized worldwide. In the last few years, new species of *Inocybe* continue to be described from around the world (Matheny & Bougher 2005, Kobayashi 2005, 2009; Jacobsson & Larsson 2009, Vauras & Kokkonen 2009, Kobayashi & Onishi 2010, Kropp & Albee-Scott 2010, Kropp et al. 2010, Kokkonen & Vauras 2011, Vauras & Larsson 2012).

In East Asia, more than 140 species of *Inocybe* have been recorded in Japan (Kobayashi 2002, 2005, 2009). This number is still increasing (Kobayashi & Onishi 2010). At present, there are ninety-eight names of *Inocybe* reported from China (Fan & Bau 2010). Unfortunately, many of the names recorded in China lack detailed descriptions or information on voucher specimens. Although a few studies on species diversity of *Inocybe* have been undertaken in some provinces (Liu 1987, Zheng et al. 1985), rather few species have been originally described as new from China (Wang 1973). Monographic studies on species diversity of *Inocybe* in China are needed.

During taxonomic studies on *Inocybaceae* from China, we encountered two impressive species easy to recognize by their conspicuous colors in the field.

Inocybe caroticolor is proposed as new based on comparative studies of available collections. Another species is found to be conspecific with *I. olivaceonigra* after examining the holotype. It is re-described based on Chinese collections. DNA sequences of internal transcribed spacer regions (ITS) of the Chinese materials were generated and analyzed.

Materials & methods

Materials were collected in *Castanopsis* forests with a subtropical highland climate at 1900 m elevation in Yunnan Province (25°03′N 102°37′E and 25°08′N 102°44′E), and examined specimens were deposited in the Herbarium of Mycology, Jilin Agricultural University (HMJAU). Additional Chinese collections from KUN (with HKAS numbers) and authentic specimens of *I. lutea* Kobayasi & Hongo from TNS and *I. olivaceonigra* and "*Astrosporina lutea*" from Z+ZT were examined (Herbarium abbreviations follow Thiers (2012)). Macroscopic characters were described based on field notes. Microscopic features were made from slide preparations mounted in 5% KOH solution and Congo Red. Measurements of microscopic features were made at 1000× on an Nikon 80i microscope. Color notations follow Kornerup & Wanscher (1981). Measurement methods follow Kobayashi (2002, 2009).

Genomic DNA was extracted from silica-dried materials with a modified CTAB protocol (Doyle & Doyle 1987). ITS gene fragments were amplified with primers ITS1F and ITS4B (Gardes & Bruns 1993). Sequencing was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd, Shanghai, China). Five new sequences (JX025772–JX025776) have been deposited at GenBank. The new sequences were used as queries of the NCBI nucleotide sequence database using the BLASTn search algorithm.

Taxonomy

Inocybe caroticolor T. Bau & Y.G. Fan **sp. nov.** Figs 1, 2

MycoBank: MB 564971

Differs from *Inocybe lutea* by its hymenial cystidia with thicker walls, its stipe covered uniformly by caulocystidia, and its aromatic odor.

Type: China, Yunnan Province, Kunming, Heilongtan Park, under *Quercus variabilis* Blume, 26 Aug 2010, Y.G. Fan 2010126 (**Holotype**, HMJAU 23271; GenBank JX025774).

Etymology: Refers to the carrot color of the basidioma.

Basidioma small. Pileus 17–33 mm in diam, conical-convex when young, then plano-convex, dry, with a prominent obtuse umbo, covered with appressed squamules, radially arranged, fibrillose, rimulose to usually rimose at margin, margin inrolled when young, occasionally recurved when mature, orange (5A7-8) to apricot (4A7-8), squamules concolorous when young, then turning brown (5C8) to reddish brown (6C8), background orange-apricot (4A8) to ochraceous (3A6). Lamellae adnexed, moderately crowded, up to 3 mm in width, pale

Fig. 1. *Inocybe caroticolor* basidiomata (HMJAU 24614).

orange to apricot (4A7-8) when young, dirty apricot (4B7) to brown (5B7) when mature, gill edge concolorous or paler, not smooth. Stipe 30–42 × 2–3 mm, solid, pale orange (5A6) to apricot (4A7), equal with subbulbous to non-marginate bulbous base, occasionally base distinctly wider, entirely pruinose on the stipe surface, densely pruinose at upper part, longitudinally striate downwards, stipe base with whitish tomentum. Cortina absent. Context with conspicuous aromatic odor, fleshy in pileus, whitish to pale apricot (4A5), 1–3 mm in width, striate in stipe, pale apricot (4A5).

Basidiospores 6.3–9.3(–9.5) × (4.4–)4.7–6.0(–6.2) μm, Q = (1.43–)1.47–1.58(–1.61), weakly to prominently nodulose (with 7–9 nodules), yellowish brown. Basidia 24–37 × 5–9 μm, with 4 sterigmata, occasionally with 1 or 2 sterigmata, clavate, narrower downwards, at times slightly enlarged at the base, usually with yellowish pigments. Pleurocystidia 48–63 × 12–16 μm, fusiform with obtuse apices, occasionally utriform, crystalliferous at apex, base obtuse or tapering into pedicel, walls bright yellow, 2–3(–4) μm thick, hyaline inside, rarely with yellow pigments. Cheilocystidia similar to pleurocystidia, more variable, occasionally cylindric; Paracystidia abundant among cheilocystidia, clavate to obovoid, thin-walled, hyaline, mixed with basidia. Caulocystidia descending to stipe base, 48–79 × 9–14 μm, abundant, similar to pleurocystidia, but more variable in shape, usually with slender habit and thinner walls (0.8–2.4 μm); Cauloparacystidia abundant, thin-walled, fusoid to lageniform, at times apex tapered, mixed with filamentous hyphae. Pileipellis a cutis,

composed of cylindric, smooth hyphae 2.0–7.0 μm wide, regularly arranged, with oil-yellow to dark-yellow intra-hyphal pigments in upper layer, almost hyaline in lower layer. Clamp connections present in all tissues, but not at every septum.

Habitat and ecology: Singly in groups, common in roadsides near forests, all known collections under *Quercus variabilis* except for one (HKAS 38963) under *Pinus yunnanensis*. June to August in Yunnan, China.

Additional specimens examined: ***Inocybe caroticolor*** — **CHINA, Yunnan Prov.**, Kunming: Kunming Botanical Garden, alt. 1900 m, under *Quercus variabilis*, 24 Jul 2011, Y.G. Fan 2011123 (HMJAU 24614; GenBank JX025773); 27 Aug 2010, Y.G. Fan 2010126c (HMJAU 24621); 27 Aug 2010, Y.G. Fan 2010126d (HMJAU 24622); 28 Aug 2010, Y.G. Fan 2010126e (HMJAU 24623); 26 Jun 2000, X.H. Wang 977 (HKAS 36740); Qiongzhu temple, alt. 2100m, under *Quercus variabilis*, 26 Jul 2011 Y.G. Fan 2011139 (HMJAU 24615; GenBank JX025772); Wuding: Shizishan, under *Pinus yunnanensis* Franch., 18 Aug 2000, F.Q. Yu 168 (HKAS 38963).

Inocybe lutea — **JAPAN**, **Shiga Pref.** Otsu city, Ishiyama-dera, on the ground in forest, 29 Sep 1955, T. Hongo 1285 (TNS-F-237714); **Ishikawa pref.**, Tatsunokuchi Hill Park, under Fagacea forest, 22 Jul 1995, G. Kazuo (TNS-F-32353).

"***Astrosporina lutea***" — **PAPUA NEW GUINEA**, **Morobe District**, Bulolo, Manki, alt. 1400 m, under *Castanopsis* and *Lithocarpus*, 28 Mar 1972, E. Horak (ZT Myc11121 [= ZT 72/335]).

Comments: *Inocybe caroticolor* can be easily recognized in the field by its carrot color, distinct aromatic odor, and entirely pruinose stipe. Microscopically, the basidiospores are nodulose, hymenial cystidia are thick-walled, and caulocystidia descend to the base of stipe. The basidiospore outline is variable. Certain collections or individuals predominately possess weakly nodulose basidiospores (HMJAU 24615, and one basidioma from HMJAU 24614, as in Fig. 1c). However, some collections or individual basidiomata possess predominately strongly nodulose basidiospores with 7–9 nodules (as in the remaining basidiomata of HMJAU 24614, HKAS 38963, and HKAS 36740; see Fig. 1e). An intermediate phenotype also exists (HMJAU 23271, HMJAU 24621, HMJAU 24622 and HMJAU 24623; see Fig. 1d). However, the ITS sequences from HMJAU 24615: GenBank JX025772 and the strongly nodulose-spored material of HMJAU 24614: JX025773 are identical with that from the holotype (HMJAU 23271: JX025774) except for a single base difference in HMJAU 24614. Furthermore, no distinct morphological difference was found among these collections. We consequently interpret the basidiospore shape divergence as intraspecific variation.

Few nodulose-spored *Inocybe* species resemble *I. caroticolor*. *Inocybe bresadolae* Massee has a similar basidiospore shape and sweet smell, but its pileus and stipe have only an indistinct orange tint that is found only in older specimens (Hobart & Tortelli 2009). Furthermore, *I. bresadolae* has a more robust habit and a context that reddens after cutting (Massee 1904, Horak

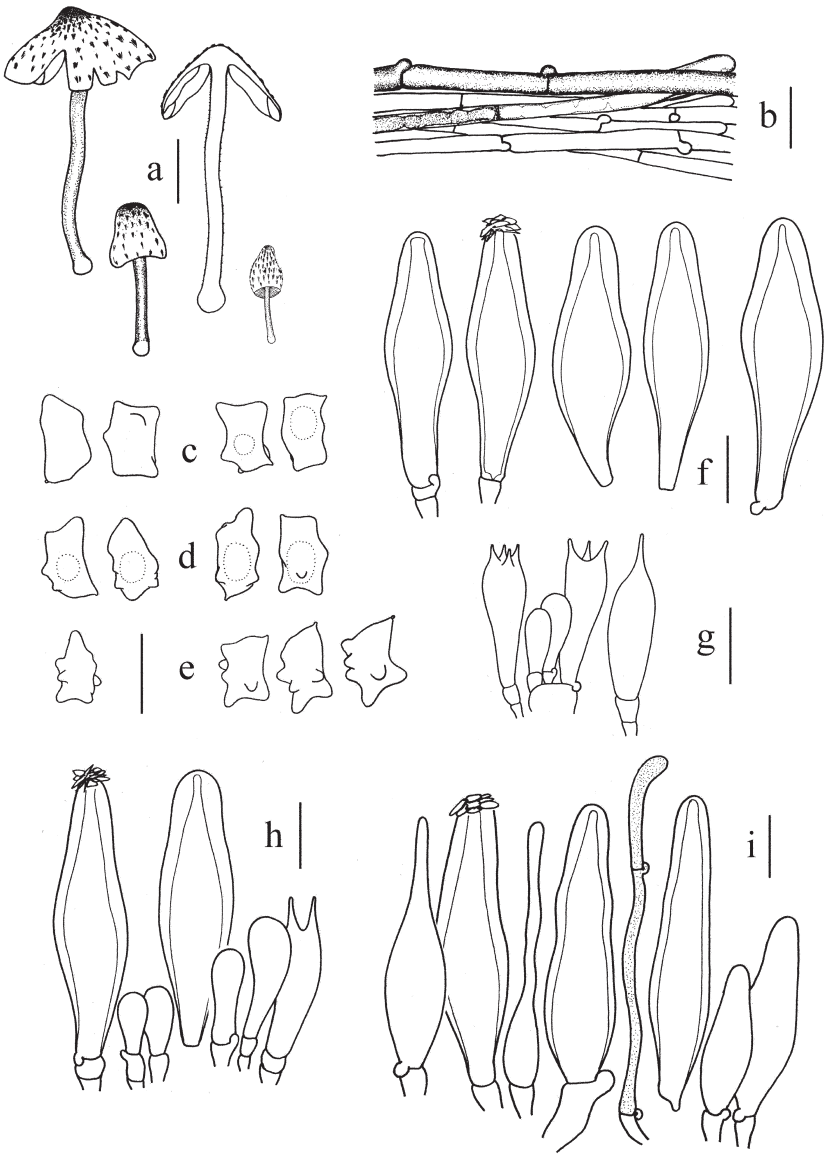

Fig. 2. *Inocybe caroticolor* (HMJAU 23271, holotype [except for c & e]). a. Basidiomata. b. Pileipellis. c. Basidiospores (HMJAU 24615). d. Basidiospores. e. Basidiospores (HMJAU 24614). f. Pleurocystidia. g. Basidia and sterile cells. h. Cheilocystidia, paracystidia, and basidium. i. Caulocystidia, paracystidia, and hypha. Scale bars: a = 10 mm, b–e = 10 μm, f–i = 20 μm.

1979). An East Asian taxon, *I. umbratica* f. *aurantiaca* Takah. Kobay., which resembles the new species to some extent, has a marginate stipe base, fungoid or grassy to spermatic odor, and its type was collected from a *Abies mariesii*, *Pinus koraiensis*, and *Betula ermanii* forest (Kobayashi 2002). *Inocybe polycystidiata* Kobayasi, described from Japan, also resembles the new species but differs by its subviscid pileus (Kobayasi 1952), recurved pileus scales (Kobayashi 2002), shorter basidia, and smaller basidiospores (Kobayasi 1952 = 5–6.5 × 4.5–5 μm; Kobayashi 2002 = 5.4–7.3 × 4.2–5.9 μm, Q = 1.1–1.5).

Another East Asian species, *I. lutea,* also resembles the new species. We have examined the authentic materials of *I. lutea* identified by Hongo (TNS-F-32353, TNS-F-237714), who helped describe the species. Our examinations reveal that it can be distinguished by the following features: (1) the pileipellis hyphae are hyaline with only a slight yellow tinge; (2) the hymenial cystidia are broadly fusiform and slightly thick-walled (1–2 μm thick); and (3) caulocystidia are present only at the stipe apex. An examination of the syntype and additional collections of *I. lutea* also reveal that caulocystidia are restricted to the stipe apex (Kobayashi 2002). Furthermore, Kobayasi (1952) originally described *I. lutea* as having a yellow fibrillose stipe that tapers upwards and an "iodine-like" odor.

Lastly, *I. lutea* as described by Horak (1979, as "*Astrosporina lutea*" (Kobayasi & Hongo) E. Horak) based on materials from Papua New Guinea is very close to *I. caroticolor* in basidioma color and entirely pruinose stipe but differs by smaller basidiospores (5.5–8 × 5–6 μm), a burnt horn smell, a marginate stipe base, and a fibrillose pileus with brown fibrils only. Our examinations of a voucher specimen of the Papua New Guinea material (ZT Myc11121) reveal pileipellis hyphae identical to those of *I. caroticolor* and hymenial cystidia that are predominately utriform usually with a truncate and strikingly thickened base. The Papua New Guinea "*Astrosporina lutea*" collections appear to share more similarities with *I. caroticolor* than with East Asian materials of *I. lutea*. However, "*A. lutea*" differs from both sets of materials at least by its unique odor.

BLASTn results of our *I. caroticolor* ITS sequences show one match (95% similarity) with unidentified environmental sequence (Wilson et al. 2008). Also, one sequence by Ryberg et al. (2008) from Estonian material labeled *I.* aff. *grammata* reaches 95% similarity.

Inocybe olivaceonigra (E. Horak) Garrido, Biblioth. Mycol. 120: 177, 1988. Figs 3–6
≡ *Astrosporina olivaceonigra* E. Horak, Persoonia 10(2): 194, 1979.

Basidiomata small, slender. Pileus 11–23 mm diam., conical at first, then convex to applanate with a blunt umbo, usually rimose or occasionally uplifted or recurved at margin in age, mostly silky-smooth, rarely scaly,

Fig. 3. *Inocybe olivaceonigra* basidiomata (HMJAU 24616).

entirely deep green (27A7-8) at first, then fading to olivaceous (28b7-8) with fuliginous tinge towards center, ochraceous (1B6-8) outwards, the olivaceous color stays at least around the center finally. Lamellae 2.5–4.5 mm broad in mid-radius, adnexed, crowded, when young cream white (1A1-2) when young, then grayish white (1B3) and discoloring to brown (4B1) with whitish edge in age; edge not smooth. Stipe 28–50 × 1.5–3.0 mm, equal with a swollen base, solid, longitudinally pruinose over the entire length except for the base, pale cream color (2A2-3), whitish at the apex and base. Context fleshy, in pileus 2.5–3.5 mm thick under the umbo, white (1A1), greenish near the pileipellis, odor spermatic; in stipe shiny, white (1A2), pinkish (5A2-3) near the surface, striate.

Basidiospores (8.7–)9.0–10.0(–10.6) × (5.8–)6.0–7.5(–7.8) μm, conspicuously nodulose, brown, Q = (1.20)1.24–1.55(1.58). Basidia 22–31 × 7–10 μm, clavate, with 4 sterigmata, clamp connections present at the base. Pleurocystidia abundant, 46–82 × 13–21 μm, thick-walled (2–3.5 μm thick), yellow, crystalliferous at apex, mostly fusiform to narrowly fusiform, at times with a short to long neck, tapered in lower part, with clamp connections at base. Cheilocystidia 41–70 × 10–18 μm, abundant, similar to pleurocystidia; Paracystidia abundant, often catenate, thin-walled, almost hyaline, terminal cells clavate to obovoid, rarely subglobose, 9–23 × 5–12 μm. Caulocystidia 37–62 × 8–21 μm, descending to the base of stipe, similar to pleurocystidia but

Fig. 4. *Inocybe olivaceonigra* in situ in a Yunnan *Castanopsis* forest.

with thinner walls; Cauloparacystidia 12–22 × 6–13 µm, obovoid or ellipsoid. Hymenial trama composed of subregularly arranged, hyaline, inflated hyphae 12–18 µm wide. Pileipellis a cutis, duplex, the upper layer indistinct pinkish in color, up to 130 µm thick, composed of subregular, cylindric hyphae 3–5 µm in diam., almost hyaline; the subtending layer golden yellow, up to 68 µm in thickness, composed of smooth to incrusted, inflated hyphae 8–20 µm in diam. Clamp connections present in all tissues, but not at every septum.

Habitat and ecology: scattered and common on the ground in forests dominated by *Castanopsis*. July to September in Yunnan, China. Type recorded from *Castanopsis* forests at high elevations in Papua New Guinea.

Specimens examined: **China**, **Yunnan Prov.**, **Kunming**: Qiongzhu temple, alt. 2100 m, under *Castanopsis*, 26 Jul 2011, T. Bau & Y.G. Fan 2011131 (HMJAU 24616; GenBank JX025775); 26 Jul 2011, X. Jin 2011131a (HMJAU 24617); 26 Jul 2011, Q.X. Guo 2011131b (HMJAU 24618); 26 Jul 2011, S.S. Yang 2011131c (HMJAU 24619); 26 Jul 2011 T. Bau 2011131d (HMJAU 24620; GenBank JX025776); 21 Sep 2006, Y.C. Li 717 (HKAS 51154). **Papua New Guinea**, **Morobe district**, Bulolo, Heads Hump, alt. 1200 m, under *Castanopsis*, 9 Mar 1972, E. Horak (ZT Myc11128 [= ZT 72/192], holotype).

Comments: The authors have examined the holotype of *Astrosporina olivaceonigra* (ZT Myc11128). The dried material is in good condition with two and a half basidiomata. The pilei are olivaceous-green around the center, paler outwards; the lamellae are apricot to yellowish brown, and the stipes are buff.

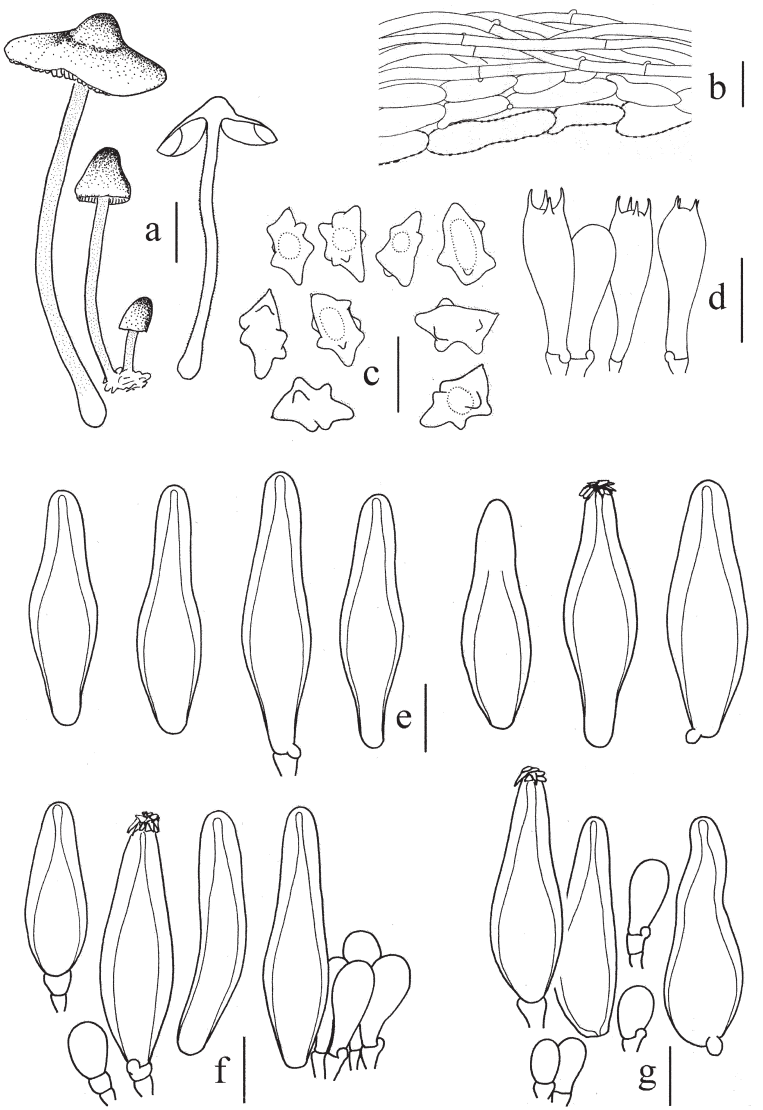

Fig. 5. *Inocybe olivaceonigra* (HMJAU 24620). a. Basidiomata; b. Pileipellis; c. Basidiospores; d. Basidia and sterile cell; e. Pleurocystidia; f. Cheilocystidia and paracystidia; g. Caulocystidia and cauloparacystidia. Scale bars: a = 10 mm; b–c = 10 μm; d–g = 20 μm.

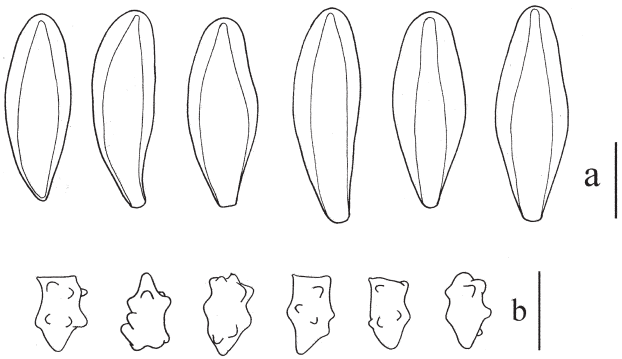

Fig. 6. *Inocybe olivaceonigra* (ZT Myc11128, holotype). a. Hymenial cystidia; b. Basidiospores. Scale bars: a = 20 μm; b = 10 μm.

Microscopically, the basidiospores (Fig. 6a) are nodulose with 9–11 rounded nodules ((7.7–)8.1–9.7(–10.7) × (5.3–)5.8–7.0(–7.8) μm, Q = (1.21–)1.34–1.55(–1.58)); hymenial cystidia (Fig. 6b) are fusoid (33–54 × 11–15 μm) with walls up to 4.5 μm; caulocystidia are similar to those of hymenial cystidia, but have thinner walls. Although Horak (1979) emphasized the conspicuous brown intra-hyphal pigments (in KOH) in cuticle hyphae in his type description, we observed that the hyphae in upper pileipellis layer in the holotype are identical to those of Chinese materials, and the subtending layer is pale yellow and composed of inflated and incrusted hyphae. The Chinese basidiospores exhibit a slightly more prominent knob at the apex, and most Chinese basidiomata possess hymenial cystidia with a thinner wall and a slender appearance (as in Fig. 5), but HMJAU 24616 and two basidiomata of HMJAU 24617 possess hymenial cystidia that agree well with the holotype (as in Fig. 6a). In view of the highly similar gross morphology, habitat, and ecology, we prefer to ignore any divergence at the species level. The two new ITS sequences (HMJAU 24616: GenBank JX025775; HMJAU 24620: JX025776) differ by a single base.

Inocybe glaucodisca Buyck & Eyssart., originally described from Zambia and which greatly resembles *I. olivaceonigra* in the glaucous grayish umbo, an entirely pruinose stipe, nodulose basidiospores, and thick-walled cystidia, differs by a finely squamulose pileus disc, a stipe base with a marginate bulb, and different habitat and ecology (Buyck & Eyssartier 1999). A recently described species, *I. pyriformis* Takah. Kobay. & S. Kurogi, which also has a smooth pileus, entirely pruinose stipe with a non-marginate base, and nodulose basidiospores differs in its dull red- to brown-colored stipe and pyriform to ventricose pleurocystidia (Kobayashi 2009). *Inocybe insignis* A.H. Sm., originally described from North

America is similar in having an entirely pruinose stipe, stellate basidiospores, and thick-walled cystidia; it shares the greenish color only after bruising or cutting. However, *I. insignis* has a larger habit, an aromatic odor, and larger stellate basidiospores (Smith 1941). *Inocybe viridiumbonata* Pegler differs by its robust habit, thin-walled cheilocystidia, slightly thick-walled pleurocystidia, and larger basidiospores. In addition, *I. viridiumbonata* occurs in a degraded xerophytic forest in the Caribbean (Pegler 1983).

BLASTn results of our *I. olivaceonigra* ITS sequences show 10 matches (94% similarity) labeled *I. suaveolens* D.E. Stuntz based on materials from North America, among which the holotype sequence of *I. suaveolens* (HQ222010) produced by Matheny is also included and three matches (93%-94% similarity) labeled *I. umbratica* Quél. based on materials from both North America and Europe. Morphologically, *I. suaveolens* (Smith & Stuntz 1950) and *I. umbratica* (Kobayashi 2002) can be easily distinguished from *I. olivaceonigra* by their lack of olivaceous colors on the pileus. The three species, however, do share the silky-smooth pileus, the entirely pruinose stipe, nodulose basidiospores, and thick-walled hymenial cystidia.

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